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PRINCIPAL INVESTIGATOR: James Cook, M.D.

CONTRACTING ORGANIZATION: University of Illinois at Chicago
Chicago, Illinois 60612

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13. ABSTRACT (Maximum 200 Words)

These studies continue to focus on defining the mechanisms by which the E1A oncogene sensitizes human tumor cells to destruction by proapoptotic, immunological and chemical injuries. Translation and testing of the key observations from an NIH-3T3 cell system to a human breast cancer cell system has been completed, and an important difference between these systems has been detected - i.e., in contrast to mouse fibroblasts, chemotherapy-induced injury of E1A-positive human tumor cells does not require expression of the wild type p53 gene. This finding has potentially important implications for the clinical relevance of the findings from this work. cDNA array studies of E1A-related differences in cellular gene expression that might be involved in the E1A-induced phenotypic change from apoptosis-resistant to apoptosis-sensitive has been initiated. Preliminary data indicate that some findings in mouse NIH-3T3 cells will be translatable into the human breast cancer cell system. A significant delay in the productivity of these studies has resulted from relocation of the research laboratory from one institution to another and the delayed transfer of funds from the original site to the new institution. As a result, the project has been extended for an additional year.

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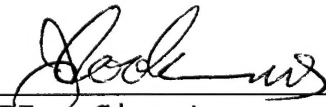
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INTRODUCTION:

These studies exploit our observation that expression of the E1A oncogene of human adenovirus types 2 and 5 converts formerly resistant cells into cells that are highly sensitive to apoptosis induced as a result of injury by killer lymphocytes, the cytokine TNF alpha and a various chemicals, including clinically relevant chemotherapeutic agents. The proposed experiments were designed to test the translation of a series of observations made during studies of mouse NIH-3T3 cells to human breast cancer cells. This comparative analysis will provide the basis for studies of the common cellular pathways, and where relevant the pathways unique to human tumor cells, that can be modulated by E1A gene expression to mediate this conversion of cells to the apoptosis-sensitive phenotype. Definition of these basic cellular mechanisms may lead to development of new strategies for enhancing the efficacy of both immunological and chemotherapeutic forms of antineoplastic therapy.

BODY:

Task 1 is to identify cellular genes whose expression is modulated by E1A protein expression to cause increased sensitivity to proapoptotic injury. It was improtant to define as clearly as possible the cellular pathway(s) through which E1A induces this cellular phenotypic change in tumor cells, to set the stage for studies of the genes involved in key cellular pathways.

It had been reported in several other cell systems that E1A-related apoptosis was dependent on cellular expression of the wild type (or native) form of the cellular transcription factor, p53 (1, 4, 6-11). These findings suggested the possibility that the mechanism(s) through which E1A induces sensitivity to apoptosis are p53-dependent. Many human malignancies, including breast cancers, have p53 mutations and are, therefore, functionally p53-negative. Therefore, it was essential to determine whether E1A-induced phenotypic conversion of tumor cells to the apoptosis-sensitive phenotype was limited by p53 mutations.

Initial studies of well-defined rodent cells were used as a starting point for comparative studies of human malignancies. This approach takesadvantage of readily available mouse cell lines and allows identification of the traits that are both common to mouse and human cells and unique to human tumor cells. By comparing p53+/+ with p53-/- mouse cells, we observed that rodent fibroblast clones expressing E1A were highly senstized to injury-induced apoptosis, independently of cellular p53 expression (2). What *was* highly dependent on p53 expression, however, was the ability of certain injuries to trigger apoptosis in sensitized, E1A-positive cells. Thus, some injuries, including most chemical injuries (including chemotherapeutic agents) and irradiation, requried p53 expression to trigger apoptosis in E1A-positive mouse cells. However, these same E1A-positive cells that could not be killed by p53-dependent chemical injuries were highly sensitive to apoptosis triggered by p53-independent injuries, including cytolytic lymphocytes and TNF alpha (2). Therefore, it appeared to be the injury initiation events, and not the E1A sensitizing mechanism(s), that depend on expression of wild type p53.

The observation that apoptosis triggered by chemotherapeutic injuries of E1A-positive mouse cells was highly dependent on p53 expression raised questions about the long-term clinical relevance of studies of E1A-induced sensitization of human tumor cells to apoptotic injuries.

Whereas definition of the molecular mechanism(s) of this E1A effect could theoretically be useful to enhance the effect of inherent or augmented immune-mediated destruction of human tumor cells, it was possible that these same E1A effects would not be useful for sensitization of p53-negative or p53-mutant human tumor cells treated with chemotherapeutic agents. To test this directly, studies of the effects of immune-mediated and chemically-induced apoptosis were repeated using human breast cancer cells (Appendix Figs. 1-4). These studies showed that human, ductal breast cancer cells stably expressing E1A oncoproteins were highly susceptible to apoptosis induced by immune injuries – human NK cells (Fig. 1), TNF-related apoptosis inducing ligand (TRAIL) (Fig. 2) and Fas antigen crosslinking (Fig. 3) – and the chemotherapeutic agent, etoposide (Fig. 4). It had been reported the these breast cancer cells may contain a mutant p53 gene. Therefore, these data suggested the possibility that, unlike the results of studies with rodent fibroblasts, chemotherapeutic agents can trigger apoptotis in E1A-positive human tumor cells irrespective of their p53 status. To test this directly, we assessed the effects of E1A expression on the apoptosis sensitivity of human osteosarcoma cell line, Saos-2, that is known to lack expression of any p53 (5) (Appendix Fig. 5). In contrast to our published observations with rodent fibroblasts (2), several different types of chemotherapeutic agents were effective at triggering apoptosis in the E1A-positive (but not the E1A-negative) cells. We had already reported that E1A expression in these Saos-2 cells rendered them highly sensitive to immune-mediated (i.e., NK cell triggered) apoptosis (3). In summary, these data revealed an important, species-related difference in the p53-dependence of chemotherapy-induced apoptosis. The finding that chemotherapy-induced apoptosis of E1A-positive human tumor cells is not restricted by p53 mutations indicates that continued studies of the molecular mechanisms through which E1A induces sensitivity to injury-induced apoptosis in human tumor cells could lead to a better understanding of mechanisms that convert apoptosis-resistant cells to apoptosis-sensitive cells in the context of both immune-mediated and chemically-induced apoptosis. These data were presented at the Annual Era of Hope Meeting in Atlanta in June, 2000.

Initial studies of differential gene expression between control and E1A-expressing cell lines were reported in the first annual report for this grant. Limited, E1A-induced differences had been found using differential display methodology. Therefore, is was decided to switch to the much more powerful cDNA chip technology for these comparison studies. An initial list of gene differences has been generated using the Affymetrix system through a collaborative study that was started before the move of the laboratory to my new institution. Analysis of these data is in progress as is repeated experiments to attempt to confirm the observations. These screening studies will be followed with Northern analysis to test the specificity of the gene expression differences and will be compared with other studies that are underway to test differential expression of targeted gene sets using the CloneTech system. At this point, the data are not sufficiently validated to report here.

Discussion. Progress on this project has been limited during the the move of my research laboratory from my previous institution to the University of Illinois Medical Center in Chicago. As noted, the move and the delay in transfer of funds has resulted in approximately an 8 month dealy in returning the project to full activity, as acknowledged by the one-year extension that was requested and granted. Despite this delay, we have made some key advances in developing and understanding the experimental system to translate information from the mouse cell model to one using human breast cancer cells. Clarification of the differences in the roles of p53 gene

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expression in human and mouse cells will be important for our studies of differential gene expression and control in the E1A-induced conversion of tumor cells to an apoptosis-sensitive phenotype. Our successful initiation of the cDNA array experiments to generate an initial list of gene expression differences between E1A-positive tumor cells and E1A-negative controls will open new areas of study of the molecular mechanisms of this E1A-induced phenotypic conversion. We will now be able to define new hypotheses regarding the p53-independent cellular pathways through which the E1A oncogene modulates the cellular response to proapoptotic injury. All of this work is consistent with the originally proposed scope of work.

KEY RESEARCH ACCOMPLISHMENTS:

- Determined that p53 mutation or deletion does not limit chemotherapy-induced apoptosis of E1A-expressing human tumor cells. This observation defines an important difference between the NIH-3T3 mouse cell system studied previously and the human ductal cell breast carcinoma system that is the focus of these studies.

- Began studies of gene expression differences between E1A-positive human tumor cells and E1A-negative control cells to provide information useful for hypothesis generation and testing regarding E1A-induced cellular pathways that control the tumor cell response to proapoptotic injury.

REPORTABLE OUTCOMES:

1. Cook, JL, Routes, BA, Walker, TA, Colvin, KL and Routes, JM (1999). E1A oncogene-induced cellular sensitization to immune-mediated apoptosis is independent of p53 and resistant to blockade by E1B 19 kD protein Experimental Cell Research **252**: 199-210.

2. Cook, JL, Routes, BA, Leu, CY, Walker, TA and Colvin, KL (1999). E1A oncogene induction of susceptibility to killing by cytolytic lymphocytes through target cell sensitization to apoptotic injury. Experimental Cell Research **251**: 414-423.

3. Cook, JL, Colvin, KL, Routes, BA and Routes, JM (2000). E1A oncogene sensitization of breast cancer cells to apoptotic injury. Poster presentation. Era of Hope Symposium, Atlanta, GA

CONCLUSIONS:

The results obtained during year 2 clarify the differences between human tumor cells and mouse NIH-3T3 cells that will be important to understand in defining the general and species-specific cellular pathways through which the E1A oncogene sensitizes cells to proapoptotic injury. These results are consistent with the original hypothesis that E1A oncogene expression converts apoptosis-resistant cells of multiple types to apoptosis-sensitive cells, but also provides clarification about the cellular pathways through which proapoptotic injuries are triggered. Neither immunological injuries nor those inflicted by chemotherapeutic agents require expression of the wild type p53 gene in human tumor cells to induce apoptosis in E1A-positive

cells. In contrast, apoptosis triggered by chemotherapeutic injuries, but not by immunological injuries, is restricted to cells expressing wild type p53 in the NIH-3T3 mouse system. Therefore, it has been determined that it is the nature of the injury and not the sensitizing effect of E1A that depends on p53 expression. This is of both theoretical and possibly practical importance, since the absence of a requirement for expression of normal p53 for triggering apoptosis in sensitized cells increases the potential application of the findings of this work to tumor cells with p53 mutations – a relatively common problem in breast malignancies.

"So what?" — The long-term goal of this project remains unchanged – to use the adenoviral E1A oncogene as a tool to identify cellular pathways and molecular mechanisms that can be used to convert chemotherapy- and immunotherapy-resistant breast cancer cells into cells that are more sensitive to these therapeutic interventions. The likelihood of being able to use the information from these studies for a variety of types of breast cancer (and other tumor) cells has been increased by the finding that the cells being targeted do not have to express a normal p53 gene. Mutations in this cellular gene is one of the most common findings in human malignancy. We will continue to focus our studies on transcriptional regulatory mechanisms through which E1A alters the cellular response to apoptotic injury, since almost all know activities of E1A involve regulation of transcription. This is likely to be the highest yield mechanistic focus around which to design studies of differential gene expression in cells that have been converted to the apoptosis-sensitive phenotype.

REFERENCES:

1. **Chiou, S. K., L. Rao, and E. White** 1994. Bcl-2 blocks p53-dependent apoptosis Mol Cell Biol. **14:**2556-63.
2. **Cook, J., B. Routes, C. Leu, T. Walker, and K. Colvin** 1999. E1A oncogene-induced cellular sensitization to immune-mediated apoptosis is independent of p53 and resistant to blockade by E1B 19 kD protein Experimental Cell Research. **252:**199-210.
3. **Cook, J., B. Routes, T. Walker, K. Colvin, and J. Routes** 1999. E1A oncogene induction of susceptibility to killing by cytolytic lymphocytes through target cell sensitization to apoptotic injury Experimental Cell Research. **251:**414-423.
4. **Debbas, M., and E. White** 1993. Wild-type p53 mediates apoptosis by E1A, which is inhibited by E1B Genes Dev. **7:**546-554.
5. **Diller, L., J. Kassel, C. Nelson, M. Gryka, G. Litwak, M. Gebhardt, B. Bressac, M. Ozturk, S. Baker, B. Vogelstein, and S. Friend** 1990. p53 functions as a cell cycle control protein in osteosarcomas Mol. Cell. Biol. **10:**5772-5781.
6. **Lin, H. J., V. Eviner, G. C. Prendergast, and E. White** 1995. Activated H-ras rescues E1A-induced apoptosis and cooperates with E1A to overcome p53-dependent growth arrest Mol Cell Biol. **15:**4536-44.
7. **Mymryk, J. S., K. Shire, and S. T. Bayley** 1994. Induction of apoptosis by adenovirus type 5 E1A in rat cells requires a proliferation block Oncogene. **9:**1187-93.
8. **Querido, E., J. G. Teodoro, and P. E. Branton** 1997. Accumulation of p53 induced by the adenovirus E1A protein requires regions involved in the stimulation of DNA synthesis J Virol. **71:**3526-33.

9. **Rao, L., M. Debbas, P. Sabbatini, D. Hockenbery, S. Korsmeyer, and E. White** 1992. The adenovirus E1A proteins induce apoptosis, which is inhibited by the E1B 19-kDa and Bcl-2 proteins Proc Natl Acad Sci U S A. **89:**7742-7746.

10. **Sabbatini, P., J. Lin, A. J. Levine, and E. White** 1995. Essential role for p53-mediated transcription in E1A-induced apoptosis Genes Dev. **9:**2184-92.

11. **White, E., P. Sabbatini, M. Debbas, W. S. Wold, D. I. Kusher, and L. R. Gooding** 1992. The 19-kilodalton adenovirus E1B transforming protein inhibits programmed cell death and prevents cytolysis by tumor necrosis factor alpha Mol Cell Biol. **12:**2570-80.

Figure 1

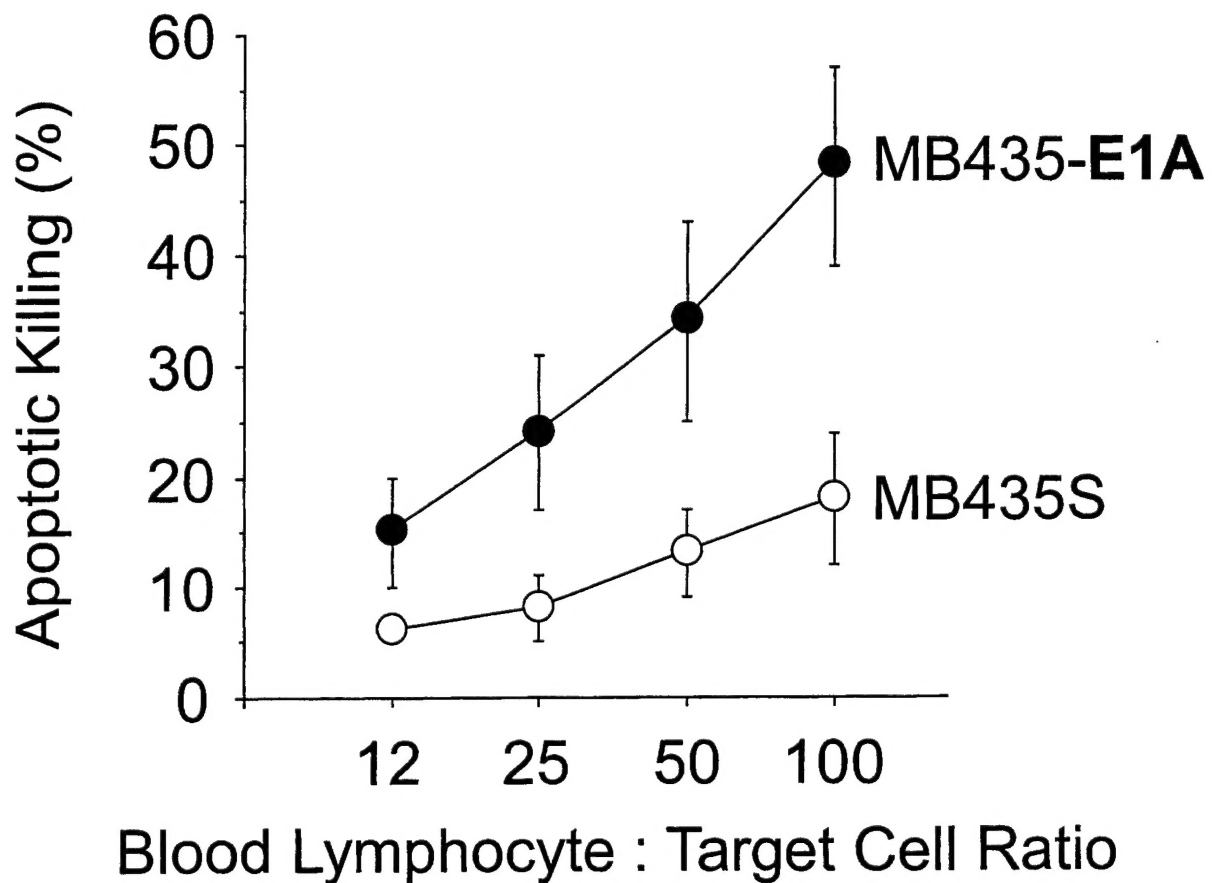# NK Cell Killing of
E1A-Positive Breast Cancer Cells

Figure 2

TRAIL Killing of E1A-Positive Breast Cancer Cells
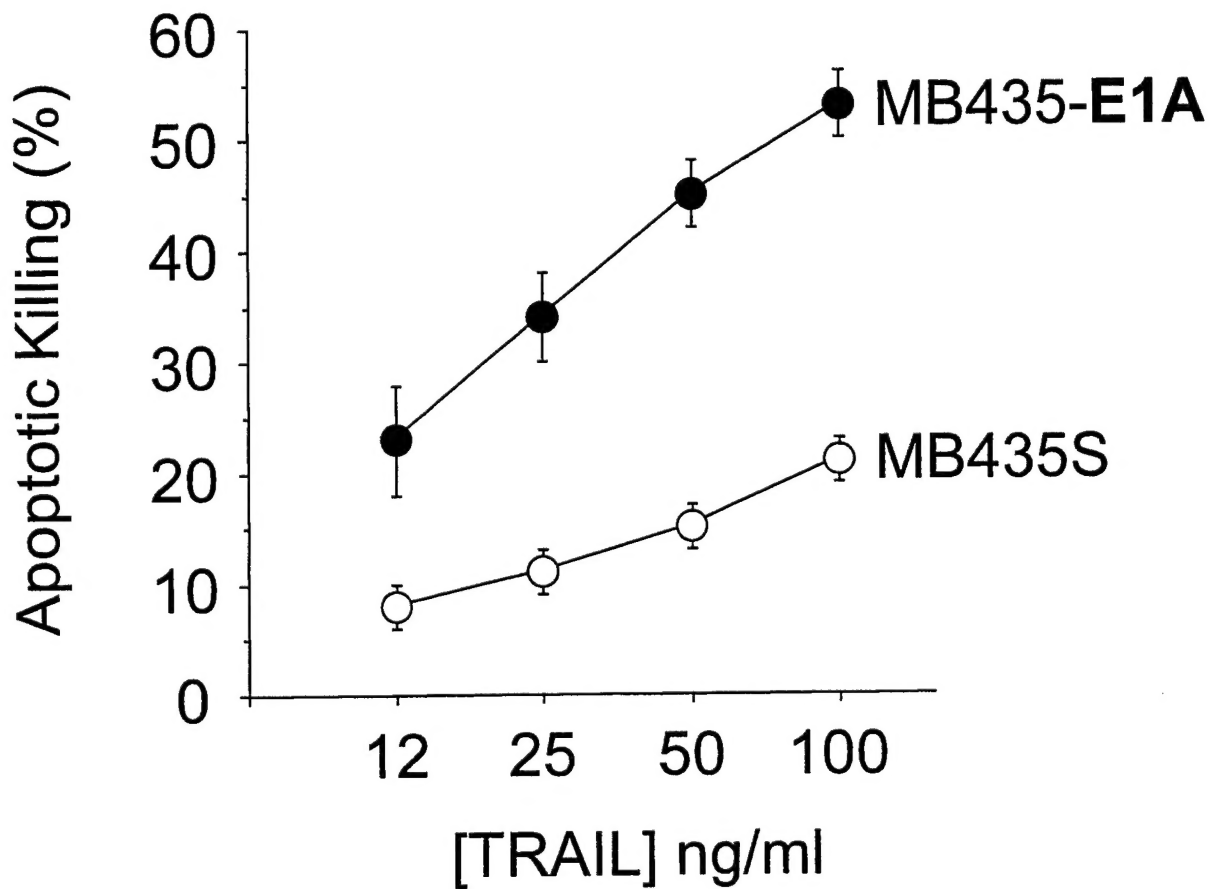
Figure 3

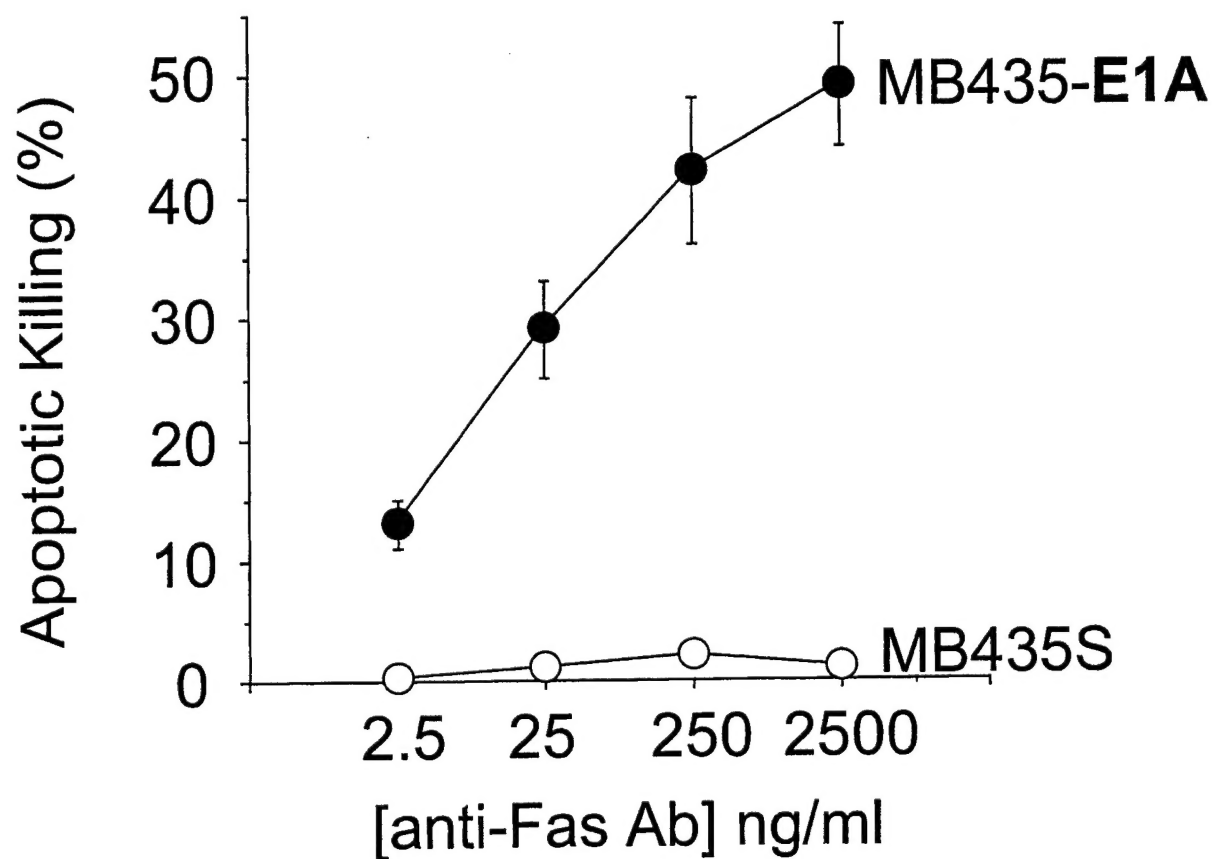# Anti-Fas Antibody Killing of E1A-Positive Breast Cancer Cells

Figure 4

Etoposide Killing of
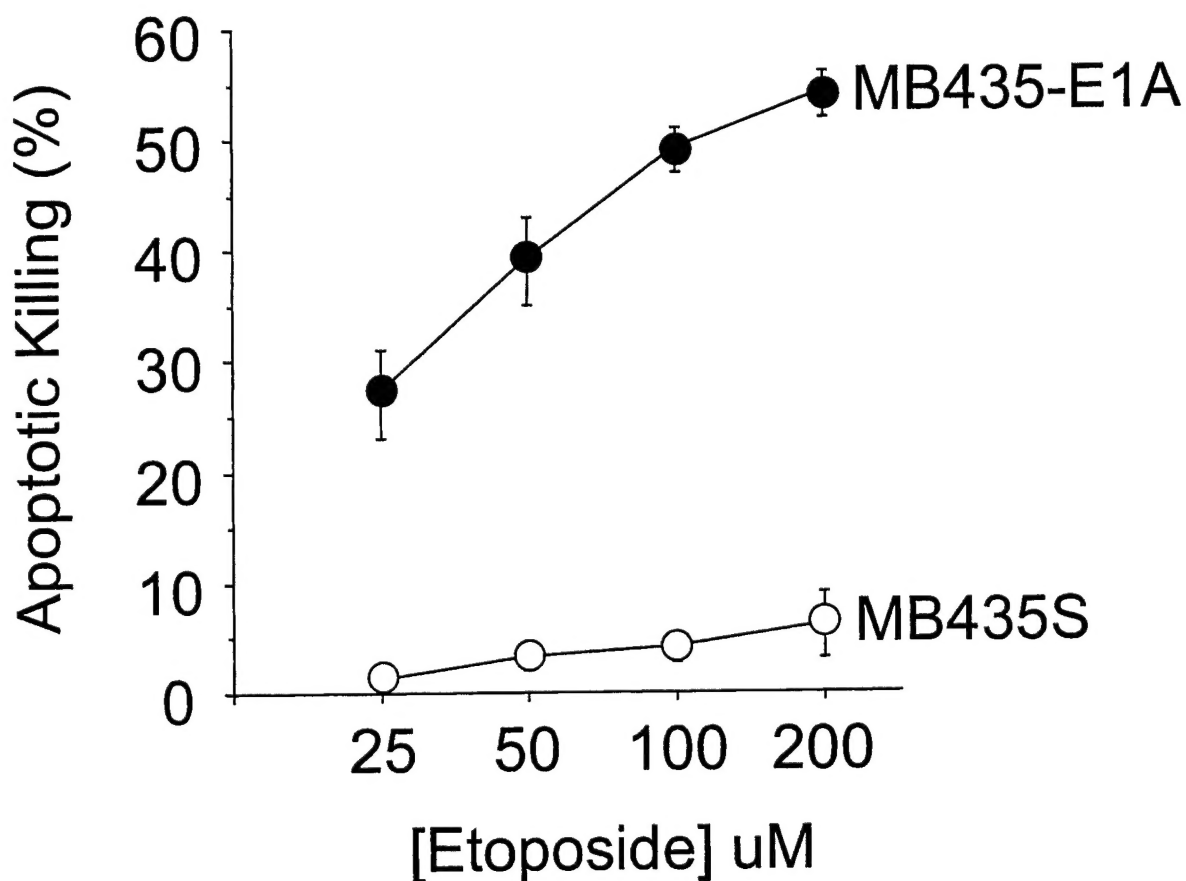# E1A-Positive Breast Cancer Cells

Figure 5

Chemotherapeutic Agent-Induced Apoptosis of **E1A+**, **p53-Negative** Human Tumor Cells

□ Saos-**E1A**
□ Saos-2